This difference in coordination style may explain the difference in cation behavior in the two series. Thus the larger Ba^{2+} ion, ineffective in the MtCRPc series, can dimerize the porphyrin whose inter-crown distance can be expanded by further rotation of the porphyrin rings, without weakening the porphyrin–porphyrin π–π stabilization. In the MtCRPc case, expanding the crown–crown distance will weaken the Pc–Pc interaction.

It is also interesting that Ca^{2+}, which does not dimerize the crown porphyrin and which forms a 1:1 (internal) adduct with 15-crown-5, is, nevertheless, able to generate a cofacial bridged MtCRPc dimer. It is evident that the free energy gained by forming a 1:1 crown ether complex is exceeded, in this case, by forming a 1:2 Ca^{2+}:crown species and the cofacial dimeric $[MtCRPc]_2[Ca^{2+}]_4$.

Lastly we would like to stress the importance of the eclipsed cofacial dimers realized in the present study. Although several covalently bound cofacial porphyrin[39] and phthalocyanine[27] dimers have been reported, they are all skewed[39] and/or staggered.[7,27] While not yet proven by X-ray studies, it is likely from the data presented here that these cofacial crown MtCRPc phthalocyanines are perfectly eclipsed and will therefore be archetypes for future studies in this area.

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Pattern of OH Radical Reaction with N^6,N^6-Dimethyladenosine. Production of Three Isomeric OH Adducts and Their Dehydration and Ring-Opening Reactions

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Abstract: By use of pulse radiolysis with optical and conductance detection, the reactions in aqueous solution of OH radicals with N^6,N^6-dimethyladenosine (DMAdo) were studied. OH reacts with DMAdo with the rate constant 6.4×10^9 M^{-1} s^{-1} by addition to C-4 (35% probability), to C-5 (19%), and to C-8 (30%) and by H abstraction from the methyl or ribose groups (16%). The resulting OH adducts A-4-OH and A-5-OH on the one hand and A-8-OH on the other undergo unimolecular transformation reactions characterized by different rates (at 20 °C) and activation parameters. With A-4-OH and A-5-OH, the transformations involve OH^- elimination (dehydration) ($k = (4.2–4.9) \times 10^5$ s^{-1}) to yield the radical cation $DMAdo^{\bullet+}$; with A-8-OH, opening of the imidazole ring occurs ($k = 9.5 \times 10^4$ s^{-1}). $DMAdo^{\bullet+}$ oxidizes *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine with $k = 2.9 \times 10^9$ M^{-1} s^{-1}. The OH^- elimination reactions of A-4-OH and A-5-OH are inhibited by protonation of the radicals, which occurs at pH 4–5 and probably involves N^6 as the proton acceptor. The elimination of OH^- is prevented also by OH^-. In contrast, the ring-opening reaction that A-8-OH undergoes is enhanced by OH^-. A-4-OH, A-5-OH, and A-8-OH and their transformation products differ also with respect to their redox properties. A-4-OH has a low reactivity with O_2, whereas A-5-OH and A-8-OH are extremely reactive.

It is well established that the interaction of ionizing radiation with living tissue causes severe damage and that the major target is the DNA of the cell nucleus. The most important types of the multiple radiation-induced lesions that occur in DNA are strand breaks, cross-links, and modifications of sugars and of bases. A particularly reactive species, which is to a considerable extent responsible for this damage, is the OH radical, one of the major primary products from the radiolysis of water which, of course, is always present in living cells.

OH radical reactions with the nucleic acid bases[2] and their mononucleosides and nucleotides have been very thoroughly studied over the past 3 decades by a variety of physical and chemical techniques.[3] An important result of these studies is that the OH radical reacts predominantly by addition to a double bond. In the case of the pyrimidines the C(5)–C(6) bond has been identified as the site of addition, and a mass balance that accounts quantitatively for the fate of the OH radical was obtained by a "redox titration" of the isomeric radicals produced.[4,5] However,

(1) On leave from Instituto Superior Tecnico, Lisbon, Portugal. The work is in partial fulfillment of the Ph.D. requirements.

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with the purines the situation is less well understood. Oxidizing and reducing radicals have been observed as products from the reaction of OH with guanosine[6,7] and adenosine,[6a,8] but their identification is considerably less straightforward than in the case of the pyrimidines. An additional complication is the fact that the OH adducts undergo unimolecular transformation(s),[3a,8-11] which have been assigned to opening of the imidazole ring of the purine[10] or, alternatively, to a dehydration reaction.[8] The latter suggestion[8] was based on the observation[4,5,12-14] that dehydration reactions typically result in redox inversion,[15] i.e., in dehydration reactions reducing radicals are converted into oxidizing ones. However, in the case of the purines the criterion of redox properties is not an unambiguous one, since assignment of the radicals in terms of oxidants or reductants is not a priori obvious and a *change* in redox character may not only be caused by dehydration but it may also result from ring-opening reactions. In view of these limitations of the method of using differences in redox behavior[16] to identify isomeric radicals, it was decided to take a different approach and look in addition at the effects on the rates of the rearrangement reactions of changing (a) a substituent and (b) the temperature and also (c) determine by conductance measurements the acid–base properties of the radicals. It is especially in this respect that use of a peralkylated adenine such as DMAdo is of advantage: a dehydration reaction should show up as elimination of OH^-, whereas with adenine or adenosine dehydration yields a nonconducting water molecule. From the results obtained it is evident that both dehydration and ring-opening reactions occur and that these originate from different radical isomers.

Experimental Section

The reagents used were analytical-grade N^6,N^6-dimethyladenosine (DMAdo) (Sigma), *tert*-butyl alcohol, $K_2S_2O_8$, $K_3Fe(CN)_6$ (Merck), and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine. HCl (Fluka) was used as received. The corresponding tetraethyl derivative was prepared (as the ethanesulfonate salt) by reacting *N,N*-diethyl-*p*-phenylenediamine (Merck) with $(C_2H_5)_2SO_4$. The aqueous solutions (using water purified with a Millipore Milli-Q system) typically contained 0.4 mM DMAdo, and they were saturated with N_2O in order to convert e_{aq}^- into OH. For experiments aimed at scavenging radicals with O_2, the solutions were saturated with mixtures of N_2O and O_2 or with pure oxygen. The pH of the solutions was adjusted with $HClO_4$ or NaOH.

The pulse radiolysis experiments were performed with a 3-MeV van de Graaff accelerator that delivered electron pulses of 0.1–0.4 μs duration with doses such that 1–2 μM radicals were produced. The optical and conductance (from an AC or a DC instrument) signals were digitized by a Biomation 8100 transient recorder interfaced with a VAX 11/38 computer via a PDP 11/10. The digitized data were stored and analyzed with the VAX. With optical detection, dosimetry was performed using N_2O-saturated 10 mM KSCN solutions for which $G(OH) = 6.0$ and $\epsilon[(SCN)_2^{\bullet-}]$ at 480 nm = 7600 M^{-1} cm^{-1}.[17] For experiments with (ac and dc) conductance detection two dosimetry systems were used: (a) a N_2O-saturated 10 mM solution of dimethyl sulfoxide[18] for which $G(H^+) = 6.0$[19] (assuming 35 cm^2 Ω^{-1} for the molar conductance of the sulfinate ion); (b) a N_2O-saturated solution at pH ≈11.2 that contained 1 mM phenol, for which $G(OH^-)$ was found to be 6.0 compared with solution (a) at the same pH, assuming a molar conductance for the phenolate ion of 35 cm^2 Ω^{-1}. The yield of radical cation from the reaction of $SO_4^{\bullet-}$ with DMAdo was calibrated by comparison with that from $SO_4^{\bullet-}$ with anisole (=100%[20]).

With the optical measurements the solutions could be thermostated to ≤0.1 °C with cells that are an integral part of a heat exchanger.

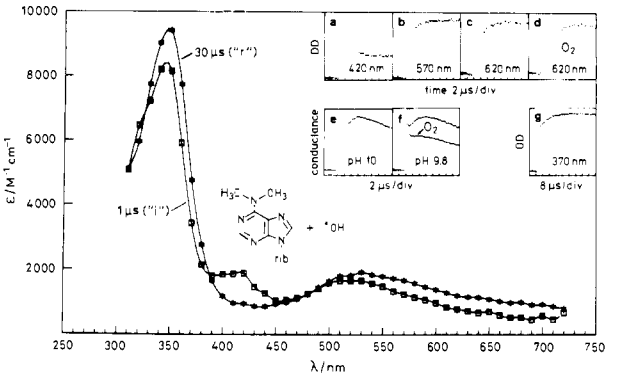

Figure 1. Time-dependent absorption spectra observed on reaction of OH with 0.4 mM DMAdo at pH 7.5 and 20 °C, $[N_2O] = 20$ mM. Key: (□) 1 μs after pulse; (★) 30 μs after pulse. The ε values were calculated assuming G(adducts) = 6.0. Insets: Changes in the OD or conductance of the solution after production of the adducts. Trace d shows the OD increase at 620 nm in the presence of 0.2 mM O_2; trace c was recorded in the absence of O_2. In the case of inset f, the lower trace was recorded in the presence of 0.2 mM O_2, the upper one in its absence. Traces a–f were recorded with 2 μs/division; trace g was recorded with 8 μs/division.

Table I. Rate Constants for Reaction of OH and SO_4^- with Substituted Adenines at pH 6–7 and 20 °C

substrate	pK_a	k/M^{-1} s^{-1} OH	k/M^{-1} s^{-1} SO_4^-
DMAdo	3.65[a]	6.4×10^9 [b]	3.9×10^9 [c]
adenosine	3.45, 12.5[d]	3.6×10^9 [e]	2.7×10^9 [c]
adenine	4.25, 9.83	4.3×10^9 [e]	4.6×10^9 [c]
9-methyladenine	3.90[f]	5.7×10^9 [b]	4.1×10^9 [c]

[a] Determined by monitoring the OD at 275 nm as a function of pH. N^6 is suggested as the site of H^+ addition. [b] This work; from the buildup of OD at 320 and 480 nm for DMAdo and at 310 and 350 nm for 9-methyladenine. The error limits are ±10%. [Substrate] = 0.02–0.4 mM. [c] From the buildup of OD at ≈350 nm. [d] Deprotonation from C'-1. [e] From ref 3a. [f] From ref 22.

Results and Discussion

(1) Formation of OH Adducts, Transformation Reactions, and Acid–Base Properties. (a) Measurements with Optical Detection. In Figure 1 are presented the spectra of the transients formed on pulse irradiation of N_2O-saturated aqueous solutions of 0.4 mM N^6,N^6-dimethyladenosine (DMAdo). The initial spectrum i is defined as that measured after completion of the reaction with OH (after 1 μs). It extends from 300 to ≥750 nm. Over this range the rates of increase of optical density (OD) after the pulse, k_{obsd}, were proportional to the concentration of DMAdo (in the range 0.05–1 mM), and from this linear dependence the rate constant for reaction of OH with the purine was obtained (at pH 7–8) as 6.4×10^9 M^{-1} s^{-1}. This value is almost a factor of 2 larger than that[3a] for reaction of OH with adenosine (see Table I). This difference in reactivity with the electrophilic OH radical probably

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Table II. Rate Constants and Activation Parameters[a] for Rearrangement of A-4-OH, A-5-OH, and A-8-OH

purine[b]	radical/reaction	k/s^{-1} (20 °C)	$k(H_2O)/k(D_2O)$[c]	E_A/kcal mol^{-1}	$\Delta S^{\ddagger}$/cal mol^{-1} K^{-1}	A/s^{-1}
DMAdo	A-4-OH/OH^- elimination	4.2×10^5[d]	2.1	9.0 (H_2O)[d]	−4.2 (H_2O)[d]	2.0×10^{12}
				9.5 (D_2O)[d]	−3.7 (D_2O)[d]	
		4.0×10^5[e]		8.7 (H_2O)[e]	−5.4 (H_2O)[e]	1.1×10^{12}
		4.5×10^5[f]				
	A-5-OH/OH^- elimination	4.9×10^5[g]		9.3 (H_2O)[g]	−2.8 (H_2O)[g]	4.1×10^{12}
	A-8-OH/ring opening	9.5×10^4[h]	1.8	4.3 (H_2O)[h]	−22 (H_2O)[h]	1.6×10^8
				4.3 (D_2O)[h]	−24 (D_2O)[h]	

[a] Determined by optical measurements at pH 6–8. [b] Concentration 0.4–1 mM. [c] Average from measurements at 10–30 °C. [d] From decay at 410–430 nm. [e] From buildup at 620 nm. [f] From conductance increase at pH 9.5–10.5. [g] From OD buildup at 560 nm. [h] From OD buildup at ≈370 nm.

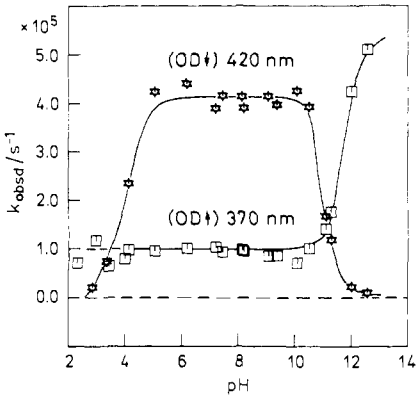

Figure 2. Dependence on pH of the rate constants at 20 °C for transformation of A-4-OH and A-8-OH, [DMAdo] = 0.4 mM. Key: (☆) decay at 420 nm; (□) buildup at 370 nm. The pH dependence of the buildup at 560 nm is similar to that of the decay at 420 nm. Below pH 4 an additional and similar buildup is seen at ≈340 nm.

reflects the larger electron density of the methylated adenine.

The initial spectrum shows maxima at ≈350, ≈420, and ≥520 nm. This spectrum undergoes time-dependent changes to give the resulting spectrum r: at ≈420 nm the optical density due to the initially formed transient(s) decreases quite rapidly in an exponential way, whereas at 330–380 and ≥510 nm the OD increases, again exponentially. However, the rates of buildup of OD in these two wavelength regions are different (see insets). The rates of all these processes were found to be independent of the concentration of (a) the substrate (in the range 0.2–2 mM) and of (b) the radicals initially produced (1–10 μM), from which it is concluded that the reactions are unimolecular. The rates are also independent of pH between ≈5 and ≈10; however, outside this range the rates change (see Figure 2). The insets a–c and g in Figure 1 show the phenomena by which the initially observed absorption spectrum i is transformed into the resulting spectrum r. The unimolecular rate of increase of OD measured at ≥510 nm was found to be $(4–4.9) \times 10^5$ s^{-1}; that of the decrease at ≈420 nm is similar, i.e., 4.2×10^5 s^{-1}, whereas the rate of increase of OD at 330–380 nm is markedly lower, i.e., 9.5×10^4 s^{-1} (see Table II).[21]

Activation Parameters of the OD Changes. In order to further characterize these processes, the activation parameters of the reactions causing the OD changes were determined by measuring the rate constants as a function of temperature in the range 0–30 °C at 5° intervals. This was done for the slow buildup at 330–380 nm, the fast decrease at 420 nm, and for the fast buildup at 570 nm and at 620 nm. From the Arrhenius plots of the rates (correlation coefficients ≥0.99) the parameters listed in Table II were obtained: the slow reaction (buildup at 330–380 nm) is characterized by a low activation energy (≈4 kcal mol^{-1}) and by a very negative activation entropy (≈−22 eu). The parameters for the decrease at ≈420 nm are quite different: the activation energy (≈9 kcal mol^{-1}) is considerably larger than that for the slow reaction; however, the activation entropy is only slightly negative (−2 to −4 eu). For this reaction, as compared with the slower one, the rate-retarding effect of the higher activation energy is overcompensated by the rate-enhancing effect of the more positive activation entropy.[21]

The activation parameters measured for the fast reaction(s) (decrease at ≈420 nm, increase at 550–600 and 610–700 nm) are equal within experimental error.

Solvent Kinetic Isotope Effects. The OD changes at 420 and at 370 nm were also measured in D_2O as a solvent. The (fast) decay at 420 nm and the (slow) buildup at 370 nm were found to be slower in D_2O by the factors of 2.1 and 1.8, respectively. From a comparison of the activation parameters measured in H_2O and in D_2O (Table II) it appears as if the solvent kinetic isotope effect is due to a change in entropy in the case of the slow reaction (buildup at ≈370 nm), whereas it results from a combination of (counteracting) enthalpic and entropic factors in the case of the fast reaction (decay at ≈420 nm).

pH Dependence. The slow and the fast reactions differ also with respect to their dependence on pH: in Figure 2 it is shown that the rate of the (fast) OD decrease at 420 nm decreases drastically with decreasing pH below pH 5 (apparent pK 4.1 for the rate retardation). A similar decrease (from 4×10^5 to $\leq 10^4$ s^{-1}) is seen when the pH is *raised* above pH 10 (apparent pK 11.0). The (fast) buildup at 560 nm has a pH dependence (not shown) similar to that of the OD decrease at 420 nm, i.e., it is decelerated in approximately the same acidic and basic pH ranges.

In contrast to the fast process, the rate of the slow OD buildup at 330–380 nm is *not* decreased below pH 4 (although the λ_{max} of the resulting spectrum changes below pH 4 from 370 to ≈340 nm, indicating that a different product may be formed). However, at pH ≥11 there is a very pronounced increase in rate (from ≈1 $\times 10^5$ to ≥5 $\times 10^5$ s^{-1} at pH 13) with an inflection point at pH 11.9.

From these different pH dependences[21] it is clear that the chemical nature of the reactions responsible for the OD changes is different. This conclusion is in agreement with that from the difference in activation parameters. Further strong support for the difference in chemical behavior of the radicals is the effect that substituents at C-6 exert on the rates of the fast and of the slow reaction: electron-donating substituents enhance the rate of the OD decrease at 420 nm very strongly ($\rho^+ = -3.0$), whereas they have only a small effect ($\rho^+ = -0.3$) on the rate of the OD increase at ≈370 nm.[22] By far the most convincing evidence for the difference between the isomers and a way to identify them comes from conductance experiments.

(b) Experiments with Conductance Detection. The reaction between OH and DMAdo results in changes of conductance, the nature of which depends on pH and on the time elasped after initiation of the reaction.[21] As shown in the inset e of Figure 1, on a fast time scale (1–6 μs after the pulse) *in*creases of conductance are seen when the reaction is carried out at pH 9–11, whereas on the same time scale *de*creases of conductance are observed at pH 4–6. In both pH ranges this fast process (see also Table II) is identical in rate with that of the fast decrease of OD at 420 nm or of the increase at ≥520 nm observed optically, indicating that with the two methods the same reactions are being monitored.

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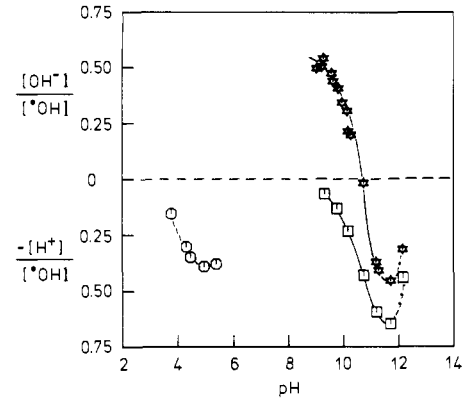


Figure 3. Dependence on pH of the time-dependent yields of OH^- produced or consumed. Key: (☆) and (○) yields measured at 5–25 μs after production of A-OH's; (□) yields measured at 100–1000 μs after production of A-OH's. Yields measured in acid solution were corrected by multiplying with only one buffering factor (1.7), i.e., that determined at pH 5.2.

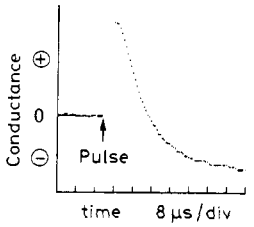


Figure 4. Conductance changes observed following reaction of OH with 0.4 mM DMAdo at pH 10.6.

If it is assumed that the fast conductance changes are due to the formation of OH^- and a radical cation of the adenine system, the yields of these ions can be calculated. Taking the molar conductance of the organic cation to be 35 and that of OH^- to be 175 $cm^2\ \Omega^{-1}$, the maximum yield of ions from DMAdo (found to be at pH ≈9.3) corresponds to 54% of the OH radicals. It is to be noted, however, that the maximal yields of the fast conductance buildup decrease with increasing pH (see Figure 3).

The hypothesis of OH^- elimination can be tested by working in acid solution. Under these conditions, OH^- elimination should lead to a decrease of conductance, since the OH^- produced removes an equivalent of H^+ from the solution. This is in fact the case, whereby the *rate* of the conductance decrease at, e.g., pH 4.3 was found to be identical with those for the fast optical changes (decrease at 420 nm and increase at ≥520 nm). However, even after correction for the buffering effect[23] of DMAdo (for pK_a values see Table I), the *yield* of the conductance change, expressed in terms of $-H^+/OH$, was found to be only 39% compared with the 54% of $+OH^-/OH$ measured at pH 9–9.3 (see Table III and Figure 3).

In basic medium, after the fast component discussed above, which leads to an increase of conductance, there was found a slower decrease of conductance (see Figure 4). At pH values above 9.5 the decrease of conductance that succeeds the initial increase became more pronounced, with respect to both rate and yield. In Figure 3 is shown the dependence on pH of the OH radical induced overall conductance changes measured after completion of (a) the fast reaction (increase of conductance) and (b) the pH-dependent "slow" reaction (decrease of conductance) and *before* the occurrence of significant radical–radical decay. In calculating the yields from the observed conductance signals it was assumed that the decrease is due to replacement of OH^- by an organic anion with a molar conductance of 35 $cm^2\ \Omega^{-1}$. It is evident from Figure 3 that the reaction with OH at pH ≥11 leads to a pronounced overall decrease of conductance, which reaches a maximum at pH ≈11.7 where it corresponds to ≈65% conversion of OH into radicals that are acidic at this pH. The *rate* of this conductance decrease was measured at pH 11.3 with 20–50 μM DMAdo, and it was found to be proportional to [DMAdo], which shows that under these conditions the rate of the reaction between DMAdo and OH determines the rate of the conductance decrease, i.e., the reaction of OH^- with the OH adducts is faster than their formation. From that dependence the bimolecular rate constant obtained for reaction of OH with DMAdo is $6.4 \times 10^9\ M^{-1}\ s^{-1}$, which is identical with the number determined from the optical experiments (see Table I).

(23) The buffering due to DMAdo (pK 3.65) was determined by measuring the conductance change in a N_2O-saturated solution containing 20 mM DMSO at pH 5.2. DMAdo (0.2 mM) was then added, which resulted in a reduction of the conductance signals by the factor 1.7. Since scavenging of OH by the purine is only 1% under these conditions, the conductance decrease must be due to buffering. The factor 1.7 was used to correct the yields of removal of H^+ at pH 5.2.

Table III. Yields[a] of Oxidized or Reduced Scavengers and Rate Constants k_s for Their Reactions[b] with A-OH or A(−H).[c] Comparison with Yields of OH^- Production

scavenger (S)	[S•+]	$k_s/M^{-1}\ s^{-1}$	[S•−]	$k_s/M^{-1}\ s^{-1}$
TMPD	53[d]	2.9×10^9	0	
TEPD	45[e]	1.9×10^9	0	
	54 (OH^-)[f]			
	39 ($-H^+$)[g]			
O_2		$\leq 4 \times 10^8$[h]		$\geq 1.6 \times 10^{10}$[i]
				$\geq 2.2 \times 10^{10}$[k]
TNM	0		37[l]	$\approx 10^9$
MV^{2+}	0		45	4.2×10^8
				1.8×10^8[m]
BV^{2+}	0		(33)[n]	1.2×10^9
$Fe(CN)_6^{3-}$		2.5×10^9[h]		8×10^9[i]

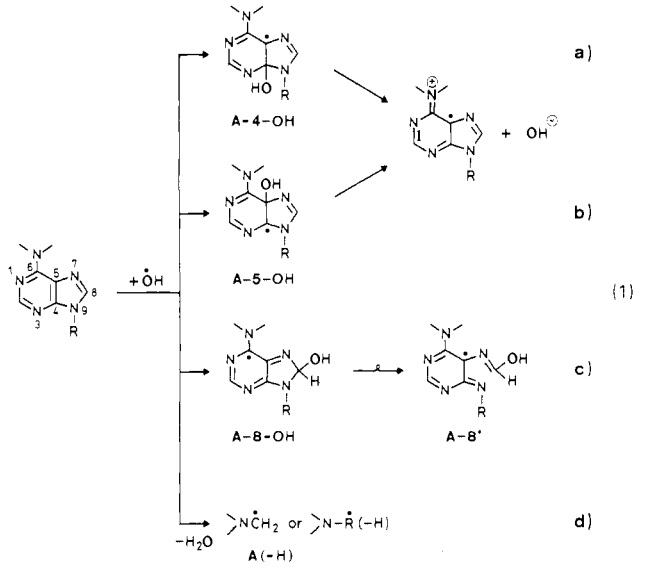
[a] In percent of •OH, determined from the plateau values of yield vs [scavenger] plots. The reference values (≡100%) are from the reaction with $(SCN)_2^{\bullet-}$ (0.2 mM TMPD or TEPD, 10 mM KSCN, see ref 4), or with $CO_2^{\bullet-}$ (0.2 mM TNM, MV^{2+}, or BV^{2+}, 0.01 M HCO_2^-. [b] [Substrate] = 0.4–1 mM, [scavenger] = 0.02–0.5 mM, 20 °C. [c] The oxidizing radicals are of type $A^{\bullet+}$ (radical cation). The reducing radicals are of type A-8-OH or its ring-opened product or those formed by H abstraction from the methyl or ribose groups. [d] pH 8–8.5. [e] pH 10. [f] From conductance at pH 9–9.3. [g] From conductance decrease at pH 5.2 (corrected for buffering of parent, see ref 23). [h] Assigned to A-4-OH. [i] Assigned to A-8-OH. [k] Assigned to A-5-OH. [l] Determined at 350 nm and corrected for the absorption of A-4-OH. [m] Measured at pH 10.5–11.7 for the slow *additional* increase of [$MV^{\bullet+}$]. The rate constant probably refers to reaction of *ionized* A-4-OH or to the ring-opened radical $A\text{-}4^-$ (see text). [n] Determined at [BV^{2+}] = 0.1 mM. A higher concentration is impractical due to the high reactivity of BV^{2+} with OH.

(2) Reaction Schemes. (a) Uncatalyzed Transformations. Since the rate of the rapid conductance change (elimination of OH^-) is the same as that of the *rapid* OD changes seen at 420, 570, and ≥610 nm, it is reasonable to assume that the two types of phenomenon are caused by the same reaction(s). The question is which radicals are responsible for these changes.

The scheme suggested, in order to explain the results, involves the assumption that OH adds to C-4, C-5, and C-8 of the adenine system to give the radicals A-4-OH, A-5-OH, and A-8-OH, and that it abstracts an H atom from the methyl or ribose groups to yield A(−H) (see eq 1). It is further proposed that A-4-OH and A-5-OH eliminate OH^- with similar, rapid rates to give rise to the same product, the radical cation of DMAdo, whereas A-8-OH undergoes opening of the imidazole ring. A(−H) is assumed not to undergo transformation reactions that lead to conductance or OD changes at λ ≥300 nm.

In the following, evidence in support of eq 1 will be discussed.

A-8-OH. Concerning addition of OH at C-8 of purines, this is well documented by product analysis studies[3a,b,e,f,i,k] in which it was shown that radicals of type A-8-OH undergo oxidation to give 8-hydroxypurines, and they also react by ring opening (reaction 1c) followed by reduction to yield 5-formamido-4,6-diaminopyrimidines as final products. Since both these types of products still contain the oxygen at C-8, elimination of OH^- does obviously not take place from A-8-OH. A-8-OH is therefore not the radical responsible for the fast reaction. A-8-OH is thus the candidate for the slow reaction (OD buildup at ≈370 nm), which



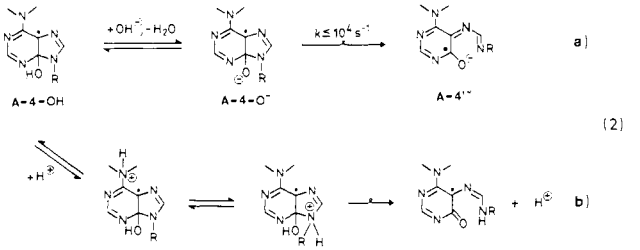
is thereby assigned to opening of the imidazole ring.

The *loss* of conductance at pH ≈9.5 at times *after* completion of the ring-opening reaction of A-8-OH (see Figure 3, squares) shows that the product of that reaction is acidic at this pH. This behavior is in agreement with an enol, but not with a formamido structure of the ring-opened radical (cf. eq 1c and 4a).

A-4-OH and A-5-OH. On the basis of the charge density distribution in purines,[24] the electron-rich C-4 and C-5 are likely additional sites for attack of the electrophilic OH radical. In comparison, the negative charge density at C-2 and C-6 is considerably smaller.[24] On this basis, attack by OH at these positions is unlikely. It is therefore suggested that it is the radicals A-4-OH and A-5-OH that are formed and that these are the ones that eliminate OH^-. That OH adds at C-4 of the purine system has also been concluded from a systematic study of the effect of substituents at C-6.[22] Further evidence for A-4-OH and A-5-OH and methods of distinguishing between the two radicals will be presented in detail in section 3.

(b) Effect of pH. From the fast conductance decrease at pH ≥ 11[25] (see Figure 3) it is evident that most of the OH adducts (as well as their transformation products) are Brönsted acids at that pH. The yield per OH of OH^- *consumed* at pH 11.7 (after 100 μs) corresponds to 65%, to be compared with a yield of 54% for OH^- *produced* at pH 9.3. In order to understand this OH^--induced inversion of apparent acidity, the following considerations are appropriate. Assuming that the 54% of OH^- produced is due to A-4-OH and A-5-OH, the remaining 46% (or less if there is H abstraction from the ribose or the methyl groups) must be due to A-8-OH. If, at pH 11.7, A-4-OH and A-5-OH still eliminated OH^- quantitatively, a net *production* of OH^- of $54 - (\leq 46) \geq 8\%$ of OH would be expected. The fact that there is an experimentally observed *loss* amounting to 65% thus indicates that at pH 11.7 A-4-OH and A-5-OH do not produce but consume to a significant extent OH^-. The mechanism that explains the pH-dependent acid–base properties of A-4-OH and A-5-OH is based on the hemiaminal or -orthoamide type nature[26] of the OH adducts. Due to the fact that OH is attached to a carbon substituted by at least one (electron-withdrawing) nitrogen, the OH group is acidic. Reaction with OH^- yields radical anions of the type $-O^-$.

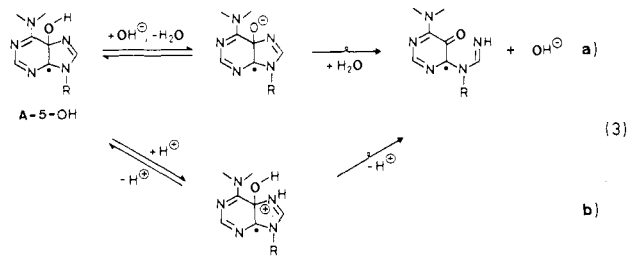
In the case of A-4-OH, its conjugate base A-4-O^- cannot possibly eliminate OH^-, i.e., elimination of OH^- from A-4-OH is inhibited by OH^-. The anion could, however, be able to undergo ring opening (analogous to nonradical hemi-orthoamides and similar tetrahedral intermediates[26]); cf. eq 2a. However, the rate



of this reaction must be $\leq 10^4\ s^{-1}$, as shown by the rate of decay of A-4-OH at high pH (Figure 2). The apparent pK of 10.8 for the OH^--induced inhibition of OH^- elimination from the conductance experiments (Figure 3) is in agreement with the pK of 11 for the change in the rate of decay of OD at 420 nm (see Figure 2), which has been assigned to OH^- elimination from A-4-OH. The OH^--induced inhibition of OH^- elimination from A-4-OH is also evident from the pH-dependent changes in the redox properties of the radicals (see section 3b and Figure 6), lending further support to the assignment.

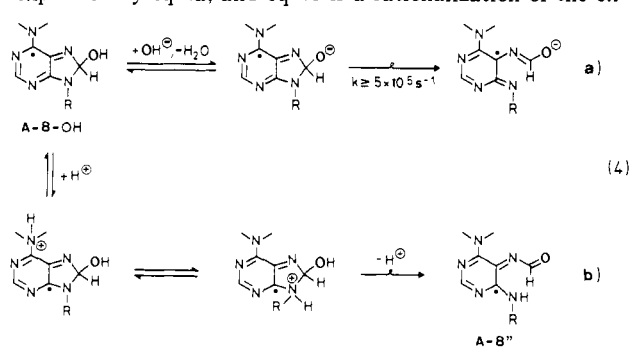
Concerning the H^+-induced inhibition of OH^- production (at pH 5.2 the yield of OH^- elimination is only 39%, compared with 54% at pH 9.3; see Figure 3 and Table III), this is suggested to involve protonation of N^6 (by which this substituent is changed into an electron-withdrawing one), followed by opening of the imidazole ring according to eq 2b. The latter reaction, which is again analogous to those[26] involving nonradical hemi-orthoamides, is proposed to be driven by protonation of N-9, followed by its deprotonation after ring opening.

Concerning A-5-OH, OH^--induced inhibition of OH^- elimination is suggested to take place in a way analogous to the case of A-4-OH. After ionization, opening of only the imidazole ring is possible (eq 3a). This would give a radical that very likely



is not ionized at pH 11.7. The same product can be formed as a result of the H^+-catalyzed inhibition of OH^- elimination, suggested to proceed via protonation of N-7 (eq 3b).

It has already been mentioned that the observed increase in acidity at pH ≈9.5 at times after ring opening of A-8-OH is indicative of the enolic nature of the product radical. The acceleration of the ring-opening reaction at pH >11 (see Figure 2) is explained by eq 4a, and eq 4b is a rationalization of the ex-



(24) Pullman, A.; Pullman, B. *Bull. Soc. Chim. Fr.* **1958**, 766. Pullman, B.; Pullman, A. *Quantum Biochemistry*; Interscience: New York, 1963; p 224.

(25) The conductance (and optical) changes observed at pH 11–12 cannot be related to the dissociation of the OH radical (pK_a 11.9) to give $O^{\bullet-}$. $O^{\bullet-}$ is usually less reactive than OH by a factor ≥10 and it tends to react by H abstraction from substituents rather than interacting with the aromatic ring (cf. ref 16). At pH >12, however, the decrease in the acidic radicals (Figure 3) may reflect the decreasing yields of OH adducts caused by conversion of OH into $O^{\bullet-}$.

(26) For reviews on solvolysis mechanisms, see: (a) McClelland, R. A.; Santry, L. J. *Acc. Chem. Res.* **1983**, *16*, 394. (b) Cordes, E. H. *Prog. Phys. Org. Chem.* **1967**, *4*, 1. (c) Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264. (d) Capon, B.; Dosunmu, M. I.; Sanchez, M. N. M. *Adv. Phys. Org. Chem.* **1985**, *21*, 37.

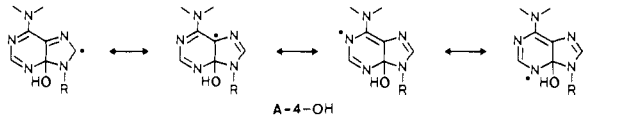
perimentally observed (Figure 2) lack of inhibition by H^+ of the slow OD increase at 340–370 nm.

(3) Redox and Scavenging Reactions of the OH Adducts. In order to further differentiate between the isomeric radicals formed by reaction of OH and to test the conclusions drawn in sections 1 and 2, experiments were performed aimed at scavenging the isomers selectively.

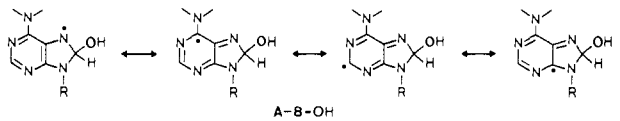
(a) Reaction with O_2. This is of particular relevance due to the ubiquitous presence of O_2 in biological systems. Organic radicals usually react with O_2 with diffusion-controlled rates,[27] and this reaction can be studied most conveniently in solutions saturated with a 4:1 mixture of N_2O and O_2, where the N_2O concentration is still sufficient to scavenge essentially all e_{aq}^- and the O_2 concentration (0.2 mM) large enough to trap organic radicals.

It was found that with DMAdo a concentration of O_2 as low as 6 μM is sufficient, at neutral pH, to completely quench the reaction that leads to the first-order increase in OD at 330–380 nm (assigned to ring opening of A-8-OH). On the basis of this observation, the rate constant for reaction of O_2 with A-8-OH can be calculated to be $k/M^{-1}\,s^{-1} \geq 9.5 \times 10^4/6 \times 10^{-6} = 1.6 \times 10^{10}$. This value is almost 10 times higher than rate constants[27] for reaction of O_2 with "normal" carbon-centered radicals.

In contrast to A-8-OH, whose rearrangement is inhibited by O_2, the fast decay of OD at 410–420 nm and the fast buildup at 620 nm (assigned to elimination of OH^- from A-4-OH) were found *not* to be influenced by O_2 in the pH range 5–10, even at $[O_2] = 1$ mM. The rate constant for reaction of A-4-OH with O_2 is

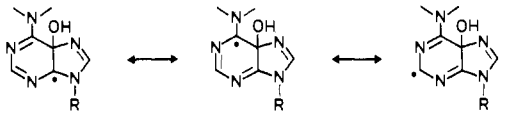


A-4-OH



A-8-OH

therefore $k/M^{-1}\,s^{-1} \leq 4 \times 10^5/10^{-3} = 4 \times 10^8$. This low reactivity is in support of the proposed structure of A-4-OH, since radicals with appreciable spin density on heteroatoms (due to delocalization of unpaired spin to N-1 and N-3) are not expected to react rapidly with O_2. In comparison, with A-8-OH there should be much less unpaired spin density on the nitrogens but more on the carbons, which should make this radical the more reactive one with respect to oxidants, in agreement with observation. Concerning A-5-OH, an even larger part of the unpaired spin density should be on carbons, and this radical should therefore be the best reductant among the A-OH radicals. On this basis, a high reactivity with



A-5-OH

O_2 is expected. In fact, the experimental evidence for the existence of A-5-OH is the effect O_2 has on the fast processes as observed by optical and conductance detection. Concerning the former, it was found that while O_2 does *not* quench the OD increase seen at >600 nm (see Figure 1, insets c and d) or the decay at ≈420 nm (both due to A-4-OH), the buildup observed at 520–600 nm (see inset b) is completely inhibited by even 22 μM O_2. As mentioned before, in the absence of O_2 ($[O_2] \leq 2$ μM), the rates of increase of OD at 520–600 nm and at >600 nm are very similar and approximately equal to that of the decrease at 420 nm (see insets a–c). Since 22 μM O_2 is sufficient to inhibit the buildup of OD at 520–600 nm ($k = 4.9 \times 10^5\,s^{-1}$), the bimolecular rate

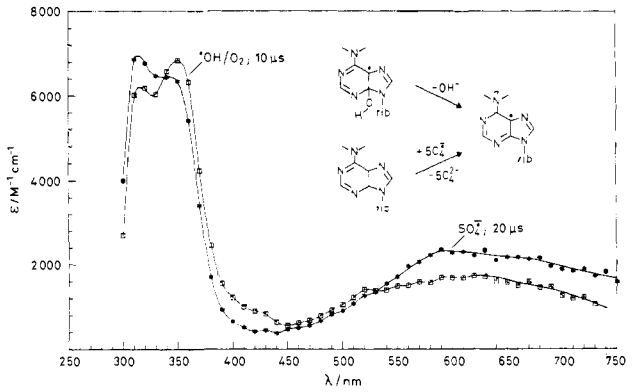


Figure 5. Absorption spectrum (★) of the DMAdo radical cation produced by reaction of 0.4 mM DMAdo with $SO_4^{\bullet-}$: $[K_2S_2O_8] = 5$ mM, 0.1 M *tert*-butyl alcohol, Ar saturated, pH 7–8, 20 °C. Comparison with the spectrum (□) recorded on reaction with OH in the presence of 0.2 mM O_2: 0.4 mM DMAdo, pH 7–8, 20 °C, $[N_2O] = 20$ mM. The ε values are based on *G* values of 3.0 for both systems.

constant for reaction of O_2 with the radical responsible (suggested to be A-5-OH) is $k/M^{-1}\,s^{-1} \geq 4.9 \times 10^5/2.2 \times 10^{-5} = 2.2 \times 10^{10}$, i.e., larger than that for A-8-OH.

In order to get an estimate of the *yield* of A-5-OH, conductance experiments were performed. It was found that by introducing 0.2 mM O_2 the maximum amplitude of the conductance increase (measured at 10–20 μs after the pulse) decreased by 20–30% (between pH 9.2 and 10.4) (see Figure 1, inset f). At pH 5, O_2 resulted in an attenuation of the conductance decrease by 40%. However, in order to translate the O_2-induced conductance decreases into yields, assumptions have to be made concerning the fate of the peroxyl radicals from A-5-OH and A-8-OH and those from the radicals formed by H abstraction from the substituents. It is likely (see later) that at least some of these peroxyl radicals $RO_2^\bullet$ undergo elimination of $O_2^{\bullet-}$ to give R^+[28] (eq 5). By this

$$RO_2^\bullet \rightarrow R^+ + O_2^{\bullet-} \quad R = \text{A-5-OH, A-8-OH, A(-H)} \quad (5)$$

reaction a part of the conductance loss due to inhibition of OH^- elimination from A-5-OH is compensated for by production of $O_2^{\bullet-}$.[29,30]

If reaction 5 did *not* exist, the 20% reduction (at pH 9.1–9.3) in the conductance increase resulting from scavenging of A-5-OH would indicate its relative concentration, i.e., 20% of the overall conductance increase of 54% of OH, i.e., 11% of OH. However, due to reaction 5, this number is a lower limit. The O_2-induced conductance decrease in basic solution is at least qualitative proof of the existence of A-5-OH: if it did *not* exist, O_2 would lead to either an increase in conductance, due to elimination of $O_2^{\bullet-}$ from A-8-OH-$O_2^\bullet$ according to reaction 5;[28] or, if A-8-OH-$O_2^\bullet$ was unable to eliminate $O_2^{\bullet-}$, O_2 would not induce any conductance change if A-5-OH did not exist. On the basis of its high reaction rate with O_2 the yield of A-5-OH can, however, be determined by differential redox titration, as described in section 3b.

Due to the differences in reactivity with O_2 between A-4-OH on the one hand and A-5-OH and A-8-OH on the other it should be possible to determine the absorption spectrum of the radical

(27) (a) Adams, G. E.; Willson, R. L. *Trans. Faraday Soc.* **1969**, *65*, 2981. (b) Willson, R. L. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **1970**, *17*, 349.

(28) Evidence for such a reaction is a rapid decay at 340–350 nm whose rate ($8 \times 10^5\,s^{-1}$) is independent of $[O_2]$ between 0.1 and 0.8 mM. For N^6,N^6,9-trimethyladenine a similar decay was seen ($k = 6 \times 10^5\,s^{-1}$). That $O_2^{\bullet-}$ is eliminated was shown by scavenging it with tetranitromethane to yield nitroform anion (NF^-): the yield of NF^- in the presence of O_2 was 10–15% less than in its absence. This difference is suggested to be due to peroxyl radicals on the ribose part of DMAdo. These radicals are not expected to eliminate $O_2^{\bullet-}$ within ≤50 μs after their formation. Cf.: Bothe, E.; Schuchmann, M. N.; Schulte-Frohlinde, D.; von Sonntag, C. *Photochem. Photobiol.* **1978**, *28*, 639.

(29) A further complication is that R^+ may react with water to produce a proton, which on neutralization would lead to a conductance decrease. There is, however, evidence from conductance experiments with ferricyanide as oxidant that the cations from A-5-OH and A-8-OH have lifetimes ≥10 ms.

(30) In acidic solution reaction 5 would affect the conductance amplitudes more (by affecting a difference) than in basic solution (effect on a sum), in agreement with experiment.

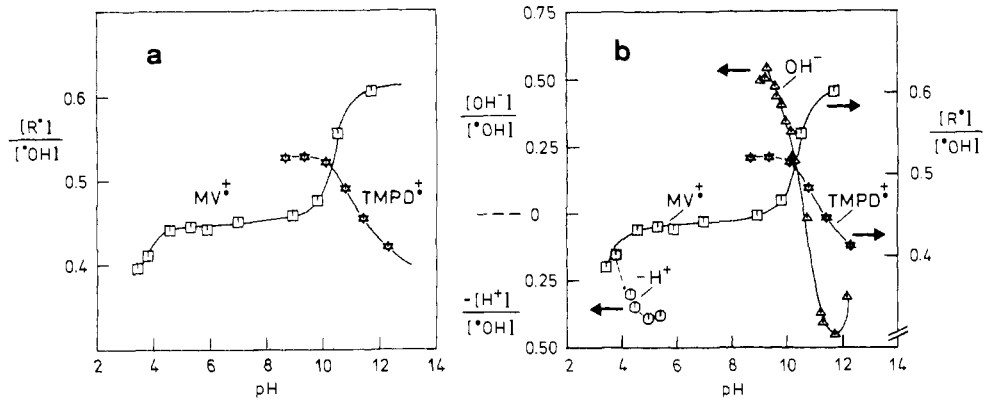


Figure 6. (a) Dependence on pH of the yields of $MV^{\bullet+}$ and $TMPD^{\bullet+}$ produced on reaction of OH with 0.5 and 1 mM DMAdo, respectively: $[MV^{2+}]$ = 0.5 mM, [TMPD] = 0.2 mM, 20 °C. (b) Comparison of (a) with the pH-dependent conductance changes (see Figure 3).

cation of DMAdo, produced by elimination of OH^- from A-4-OH in the presence of 0.2 mM O_2 to scavenge A-5-OH and A-8-OH. The spectrum recorded under these conditions is shown in Figure 5, together with that obtained on reacting DMAdo with the radical $SO_4^{\bullet-}$, which gives the radical cation, based on conductance experiments using the reaction $SO_4^{\bullet-}$ + anisole for calibration.[20] The spectra are seen to be quite similar, but not identical, as to be postulated if the peroxyl radicals produced by O_2 from A-5-OH and A-8-OH did not absorb at ≥300 nm. The difference between the spectra can, however, be qualitatively explained by a reaction by which the peroxyl radicals from A-5-OH and A-8-OH are rapidly converted into products that absorb in the region 300–700 nm. Elimination of $O_2^{\bullet-}$ to give (delocalized) cations is such a possibility, cf. eq 5.

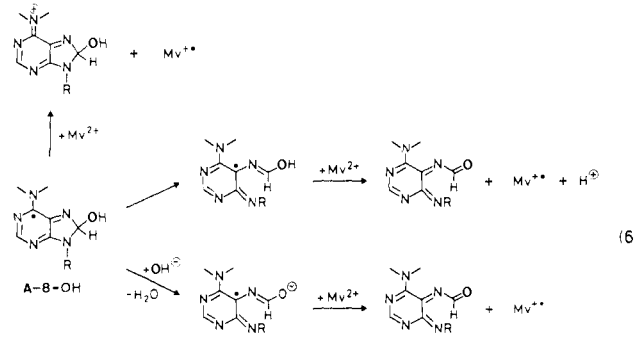
(b) Tetranitromethane (TNM), Methylviologen (MV^{2+}), *N,N,N′,N′*-Tetramethyl-*p*-phenylenediamine (TMPD), and *N,N,N′,N′*-Tetraethyl-*p*-phenylenediamine (TEPD). The oxidant TNM and the reductant TMPD have been used to differentiate between reducing and oxidizing radicals produced from pyrimidines[4,5,31] and also from purines.[6-8] In the present study N_2O-saturated 0.5 mM solutions of DMAdo in the presence of 0.02–0.5 mM TNM or MV^{2+} were pulse irradiated. After formation of the OH adducts by reaction of OH with the excess purine,[32] the production of $C(NO_2)_3^-$ (NF^-; λ_{max} = 350 nm) or of $MV^{\bullet+}$ (λ_{max} = 605 nm) was seen with solutions containing, respectively, TNM or MV^{2+}. The yields of NF^- or of $MV^{\bullet+}$ increased with the concentrations of the oxidants up to a plateau corresponding, at pH 6–8, to ≈45% of the OH radicals initially present. Also, the rates of formation of the semireduced oxidants increased with [oxidant], and from these dependences the rate constants for reaction of the reducing radicals with MV^{2+}, TNM, and benzylviologen are 4.2×10^8, 10^9, and 1.2×10^9 M^{-1} s^{-1}, respectively (see Table III).

With 0.5 mM DMAdo in the presence of 0.5 mM MV^{2+} a pH variation was performed in the range 3.5–12. Between pH 5 and 9.5 the rates and the yields of $MV^{\bullet+}$ production did not vary. From pH ≈4.5 to 3.5 both rates and yields decreased sharply; in contrast, at pH >9.5 the yields increased. Kinetically, this increase is due to a new reaction with a slower rate (Table III) that leads to a further increase of OD on top of that observed at pH 5–9. The dependence on pH of $MV^{\bullet+}$/OH measured at 605 nm after completion of the formation reaction(s) is shown in Figure 6a, which also contains the pH dependence of the oxidizing radicals.

Complementary experiments were performed using TMPD and TEPD as scavengers for oxidizing radicals. TMPD has previously been used for reactions of this kind;[4-8,31] TEPD was chosen because it is a considerably stronger reductant than TMPD.[34] The N_2O-saturated solutions studied contained 1 mM DMAdo and 0.02–0.4 mM TMPD or TEPD. Under these conditions the OH radicals are scavenged predominantly by DMAdo. The formation of $TMPD^{\bullet+}$ or $TEPD^{\bullet+}$, the radical cations of the amines, was observed, showing that purine radicals with oxidizing properties have been scavenged. The yields per OH of oxidizing radicals, based on both $[TMPD^{\bullet+}]$ and $[TEPD^{\bullet+}]$, were found to be ≈50% (see Table III), in excellent agreement with the number obtained for the sum of A-4-OH and A-5-OH from the yield of OH^- elimination measured by conductance. The rate constants for formation of the amine radical cations are (2–3) × 10^9 M^{-1} s^{-1}. A pH variation was performed in the presence of TMPD. At pH >10 the rates of production and the yields of $TMPD^{\bullet+}$ decreased drastically in a sigmoidal way, as shown in Figures 6a and b. The latter also contains the conductance data. It is obvious that OH^- inhibits the production of oxidizing radicals and instead catalyzes the formation of reducing radicals. On the basis of the similarity of the inflection points it is clear that the two sets of data (and the optical data shown in Figure 2) describe the same reactions.

In an experiment aimed at determining the individual yield of A-4-OH, a N_2O-saturated solution containing 2 mM DMAdo and 0.2 mM TMPD was irradiated, and the yield of $TMPD^{\bullet+}$ was measured (at 30 μs after the pulse) to be 54% of OH. Oxygen (0.2 mM) was then introduced to scavenge A-5-OH. This resulted in a reduction of the $TMPD^{\bullet+}$ yield to 35% of OH, with no change in the *rate* of $TMPD^{\bullet+}$ production. The radical nonscavengeable by O_2 is obviously A-4-OH (on the basis of its lack of reactivity as monitored by the OD decay at 420 nm), and the remaining 19% (to 54%) is therefore due to A-5-OH.

The pH-dependent redox properties of the radicals are explained by reactions 2–4 and 6 and 7.



Both A-8-OH and its ring-opened enolic product A-8′ are suggested to be able to reduce MV^{2+} [or $Fe(CN)_6^{3-}$] (see eq 6). This is concluded from the linear dependence of k_{obsd} for formation of $MV^{\bullet+}$ on $[MV^{2+}]$ from 0.05 mM (where ring opening predominates) to 0.5 mM (where oxidation of A-8-OH is faster).

(31) Schuchmann, M. N.; Steenken, S.; Wroblewski, J.; von Sonntag, C. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **1984**, *46*, 225.

(32) The rate constant for reaction of OH with DMAdo is 6.4 × 10^9 (Table I), that with TNM is <10^6, and that[33] with MV^{2+} is 6.4 × 10^8 M^{-1} s^{-1}. Under conditions of equal concentrations of DMAdo and oxidant, the percentage of OH reacting with the oxidant is thus ≤7%.

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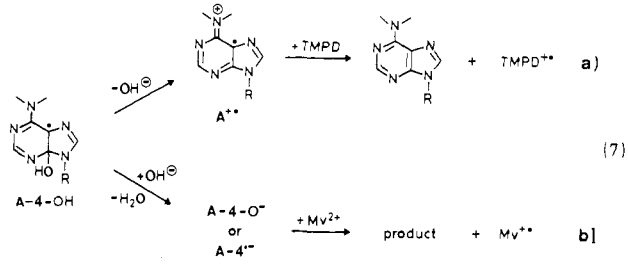
(34) Steenken, S. In Landolt-Börnstein, *Zahlenwerte und Funktionen aus Naturwissenschaften und Technik* **1985**, *13e*, 147.



(7)

Protonation is suggested to take place at N^6 of A-8-OH (eq 4b) by which the radical loses its reducing power, due to conversion of the electron-donating dimethylamino group into the electron-withdrawing aminium group. The formamido-substituted radical A-8″ formed by H^+-catalyzed ring opening (eq 4b) does not appear to be a reductant either. This explains the decrease in the rate and in the yield of reduction of MV^{2+} observed below pH 4.5 (see Figure 6).

Of particular interest is the OH^--induced inhibition of OH^- elimination from A-4-OH (reactions 2a and 7b), by which the conversion of this radical (which is a weak oxidant itself, see ref 22, note 29) into the more strongly oxidizing radical $A^{\bullet+}$ is prevented and changed into the opposite, i.e., into a radical ($A\text{-}4\text{-}O^-$ or, possibly, $A\text{-}4'^-$) with reducing properties with respect to MV^{2+}. This is a redox inversion reaction in which, in contrast to OH^--catalyzed OH^- elimination reactions,[4,5,12,13,14b] an oxidant is transformed into a reductant. The reaction sequence A-4-OH → $A\text{-}4\text{-}O^-$ ($A\text{-}4'^-$) is documented by the increase with pH of the reducing equivalents, by the complementary decrease in the oxidizing equivalents (Figure 6), by the changes with pH in the production or consumption of OH^- (Figure 3), and by the pH dependence of the rate of OH^- elimination as determined by optical detection (Figure 2).

If, as assumed in reactions 2–4, the conductance decrease at pH 11.5 (65% of OH) represents the sum of A-4-OH and A-8-OH, from the 35% for A-4-OH the yield of A-8-OH results as 30% of OH. Since the conductance increase at pH 9–9.5 (54% of OH) is due to A-4-OH and A-5-OH, the remaining 46% must be due to A-8-OH and A(−H). The same number (45%) is obtained for A-8-OH + A(−H) from the experiments with MV^{2+} (see Table III). Subtracting from this number the 30% for A-8-OH gives 15% for A(−H). This value is reasonable on the basis of the known preference[16] of OH for addition versus H abstraction.

(4) Summary and Conclusions. It has been demonstrated that the OH radical reacts with DMAdo by addition to C-4 (35%), C-5 (19%), and C-8 (30%) and that the resulting radicals undergo unimolecular transformation reactions that have been characterized by their activation parameters and pH dependences and identified as OH^- elimination (dehydration) and opening of the imidazole ring, respectively. The result of the OH^- elimination is the one-electron oxidized molecule $DMAdo^{\bullet+}$. The OH addition/OH^- elimination sequence is an example for "inner-sphere" electron transfer.

The heterolytic transformation reactions of the hemi-aminal or hemi-orthoamide type A-OH's can be accelerated or decelerated or diverted into another direction by reaction with H^+ or OH^-. For instance, with A-4-OH both H^+ and OH^- prevent the elimination of OH^-, and they instead induce a reaction that probably involves opening of the imidazole ring. In the case of A-8-OH, OH^- accelerates the rate of the uncatalyzed ring-opening reaction, whereas with H^+ the rate-accelerating effect due to protonation at N-9 is just about balanced (see Figure 2) by the rate-decelerating effect[22] due to protonation at N^6. The spontaneous and H^+- or OH^--catalyzed (heterolytic) ring-opening reactions of the tetrahedral intermediates A-OH are of a type well documented[26] for analogous nonradical systems.

Of special interest are the differences between the isomeric A-OH's with respect to reactivity with O_2. A-5-OH and A-8-OH are highly reactive. In contrast, A-4-OH has a very low reactivity with O_2. These differences in reactivity are probably related to differences in unpaired spin densities at the various ring positions, a high spin density on a heteroatom causing a low reactivity with O_2. A second factor that determines the reactivity with oxidants is the extent to which a positive charge developing at a site can be stabilized by a substituent: a charge at C-4 and C-6 is obviously stabilizable efficiently by the nitrogens N-9 and N^6. This is possible in the case of A-8-OH and A-5-OH, but not with A-4-OH. It is interesting that the difference in redox properties between A-5-OH and A-4-OH is qualitatively the same as that[4,5,31] between the analogous *pyrimidine* radicals P-5-OH and P-6-OH. A further common feature of purine and pyrimidine OH adducts is their ability to undergo dehydration. However, in contrast to the pyrimidines,[4,5] with the purines the special feature is that this reaction occurs spontaneously (uncatalyzed) and that it also takes place with the nucleosides and nucleotides.[22]

A note may be added concerning OH adducts of naturally occurring adenines. With these, the dehydration reaction is expected to lead to neutral radicals centered at N^6, as a result of deprotonation from the (incipient) radical cation.[6a,8,22] Due to high unpaired spin density at the nitrogens, these radicals are oxidizing and, therefore, can be repaired by reductants.[8]

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